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Effects of Mechanical Loading on Articular Cartilage of Young *Cavia Cobaya*: Histomorphometry and Electron Micrography

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Summary

Observing the early phase of response to mechanical load, we evaluated morphometrically articular and growth cartilage of knee joint in immature guinea pigs in comparison with unloaded controls.

Intracellular substance and articular cartilage thickness showed after initial stress increase, in further progress reduction and stagnation. Up to 11000 m an abrupt increase of intercellular substance was accompanied with decrease of cartilage thickness, cellnumber and cellvolume in tangential zone and cellvolume-reduction either in radial zone.

Diminishment of epiphyseal thickness and tibial length compared with same aged controls was remarkable after 11000 m. We suggest an adaptive potential of growing cartilage to respond mechanical stress in a range of tolerance.

Introduction

In exploration of the early phase of osteoarthritis exists a necessity to know, in which way the cartilage internal system decompensates below the level of pure, mechanical destruction under chronic exercise^{1,2)}. In this respect the study of the earliest response and the adaptive potential of growing articular and epiphyseal cartilage to mechanical stress is the purpose of our experiment. We attached great importance to the preservation of the articular integrity and the physiologic movement, which can cause, applied in certain ways, osteoarthrotic change. In other models of experimental induced degenerative changes the instability of joint for instance after section of cruciate ligaments or extirpation of meniscus in adult animals is preferred^{8,10,13,21)}, but these changes in a sense of prearthrotic deformations are irreversible, impossible to influence

Key words: Osteoarthritis, Experimental arthritis, Articular cartilage, Growth plate, Chondrocyte.

索引語: 変形性関節症, 実験的関節症, 幼若モルモット, 膝関節, 組織学的計測

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and rapidly, that the early changes in cartilage must be difficult to study. Especially the synovial inflammation may play a considerable role, which mark the different used terminology of osteoarthritis and osteoarthrosis.

Materials and Methods

We have used juvenile male albino guinea pigs; in the beginning of experiments 2 and in the end 3, 5 month old with an initial weight between 350 g to 400 g and divided into 6 randomized groups, running-groups (II, III, IV, V, VI) and control (I), each containing 12 animals. The guinea pigs had to run on a conveyer-belt without experienced painful trauma and to cover distances of 1000 m to 11000 m with speed of 10 m per minute, 200 m once a day over 7 days per week. A special apparatus in accordance to the experiment of STOFFT 1980^{22,23)} was designed, that would provide continous active motion in quadruped gait of each of 16 animals at the same time.

The mechanic drive equipment was connected and controled by an electronic automat for registration of time and intensity of load. We didn't use electric shock for stimulation to walk, to exclude any additional hormonal influences. The animals were provoked to walk by a simple sensor brush, localized in the onset of the belt, and due to the selfmovement of the belt. If one animal became uncomfortable in the apparatus and the struggle altered the load-time-record, this animal was excluded from the further experiment. The animals were sacrificed 24 hours after the last loading. Light- and electron microscopic morphometrical evaluations were undergone.

After 11000 m and 55 days the tibial length of loaded and unloaded animals were radiologically evaluated.

Histomorphometry:

Frontal sections of the whole knee joint were cut 10 microns thick and chondrocytes of tangential and radial zone of articular cartilage and proliferative zone of epiphyseal plate of femoral condyle and upper tibia were morphometrically analysed. The surface density, volumetric density, number of profiles, cellvolume, area fraction, numerical density and section thickness²⁴⁾ were evaluated. (zonal morphometric findings).

We made a point of comparison between the different loaded locations namely periphery-covered by meniscus, central-uncovered by meniscus, medicl and lateral condylus. (local morphometric findings). We examined with a statistical security of 100 evaluation-unit areas per location, a magnification of 1000 and with a testline length of 0,5 μm .

Transmission Electron Micoscopic Morphometry:

Chondrocytes of the radial zone were prepared for electron microscopic examination. 485 chondrocytes were photographed with magnification of 12000. The following parameters were documented morphometrically: area of nucleoli, number and volume of mitochondria, number of golgi-bodies, number and area of fat-vacuoles, number of lysosomes, area of

endoplasmatic reticulum-channels, dilatation and area of microfilaments. Changes in the organelle system of the chondrocytes can be documented qualitatively and quantitatively only by statistical evaluation of large groups of cells. We examined at least 70 cells per group.

Results

(Histomorphometry)

Only minute irregularities after 11000 m for instance roughness of lamina splendens, minimal flakes or splittings in comparison with the controls, in which no initial alterations occurred, could be obtained.

Zonal Morphometric Findings:

Advance of intercellular substance, coincidental advance of cartilage thickness with consequently relative decrease of cell number and of mean cellvolume in tangential zone was observed after 1000 m. In further loading the decrease of cartilage thickness and volume density of intercellular substance with still diminished number of chondrocytes was accompanied with apparent increase of mean cellvolume in 3000 m and 5400 (Fig. 1). After 8000 m the cartilage thickness returned to the controlvalue, although the volumetric density of intercellular substance was increased. Therefor we could find a reduced cellnumber and obvious diminished cellvolume in tangential zone up to 11000 m. The progress in the first step to 1000 m was in the radial zone similar in comparison with the tangential zone. When cartilate thickness and volume density of intercellular substance were returning between 3000 m and 5400 m to the controlvalue, the

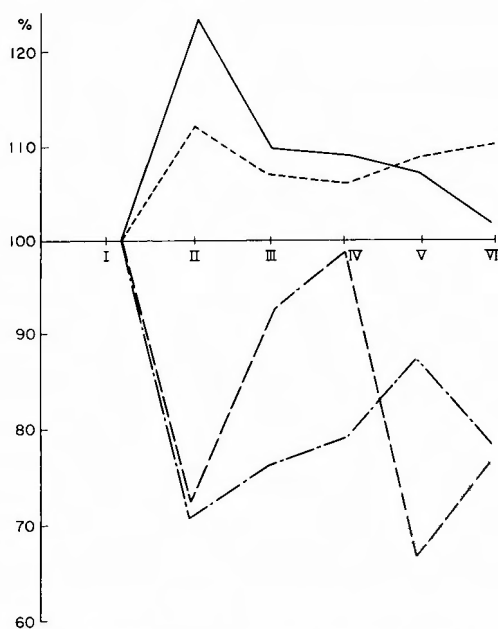


Fig. 1. progress of cartilage thickness —————; volumetric density of intercellular substance; number of chondrocytes - . - . - and mean cellvolume - - - - in tangential zone (according to 100% of controlgroup).

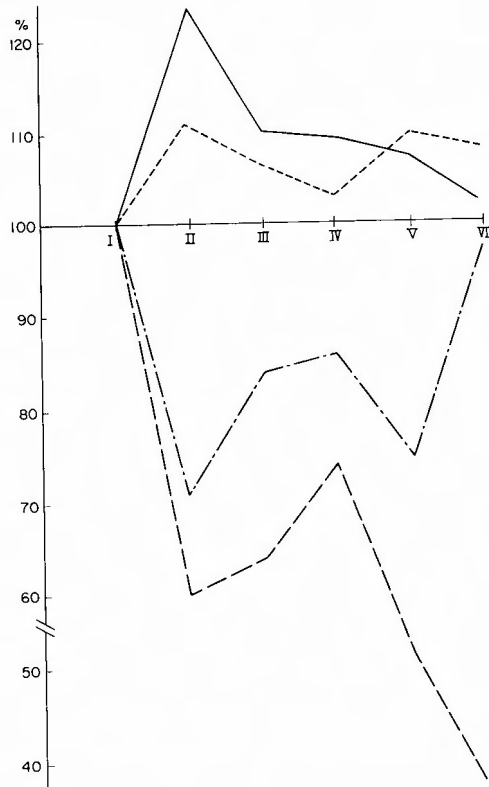


Fig. 2 progress of the same parameters as in Fig. 1 in radial zone (according to 100% of controlgroup).

cellnumber and the mean cellvolume were relative increased (Fig. 2). When the cartilage thickness was further reducing after 5400 m to the controlvalue, the volume density of intercellular substance was abrupt increased. In consequence the cellnumber was relative decreased. On the other hand the cellnumber was absolutely increasing after 8000 m accompanied with further cellvolume diminishment and further increasing of volume density of intercellular substance to 11000 m.

Local Morphometric Findings:

The percent portion of cartilage thickness increase is significant higher ($p<0,05$) in central location of medical femoral condyle uncovered by meniscus (Fig. 3). The cellnumber of tangential zone in uncovered central location of medical condyle is significant less ($p<0,05$) than in periphery of all groups. The mean cellvolume in tangential zone in uncovered medial condyle is significant higher ($p<0,05$) than in medial periphery covered by meniscus in 8000 m- and 11000 m group whereby in control—up to 8000 m-group the mean cellvolume of radial zone in uncovered medial location is significant less ($p<0,05$) than in covered medial periphery. In 11000 m-group this value has no significant difference in both locations of medial femoral condyle (Fig. 4). The number of chondrocytes in tibial likewise in femoral proliferative zone of epiphyseal

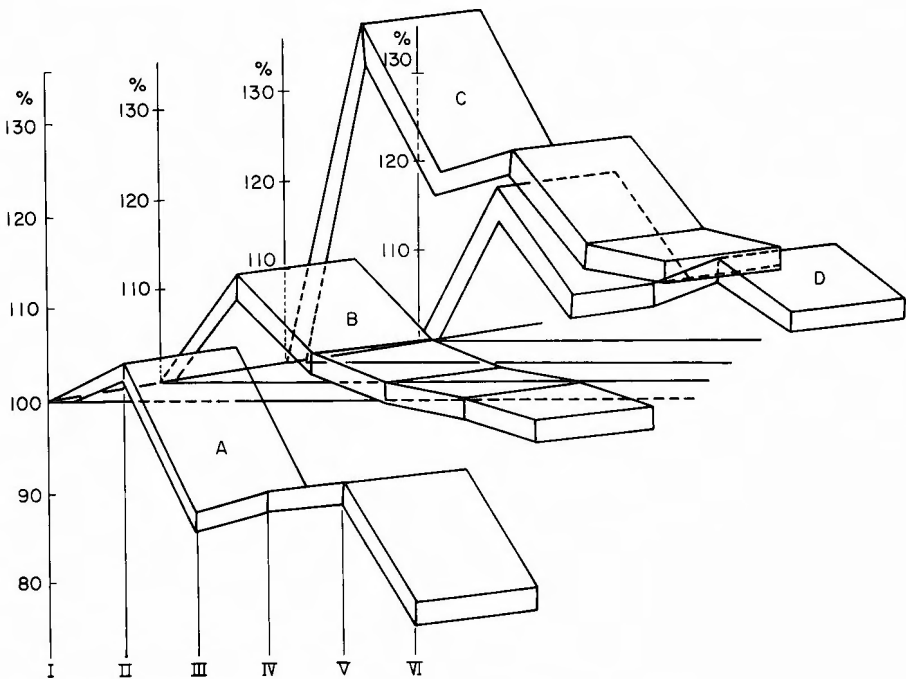


Fig. 3 progress of cartilage thickness in:
A=growth plate (proximal tibia);
B=medial peripher/meniscuscovered (femoral condyle);
C=medial central/meniscusuncovered (femoral condyle);
D=lateral peripher/meniscus covered (femoral condyle)—(according to 100^o of controlgroup).

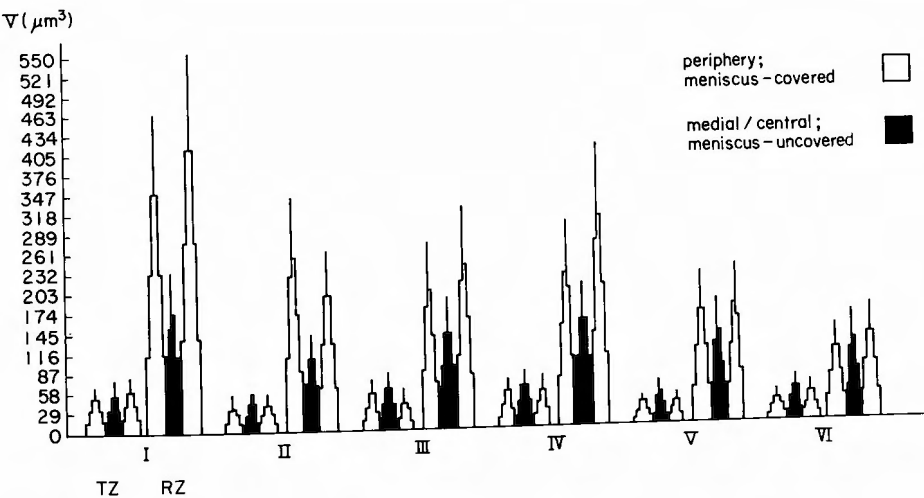


Fig. 4. Mean cellvolume in tangential- and radial zone in comparison with evaluated locations

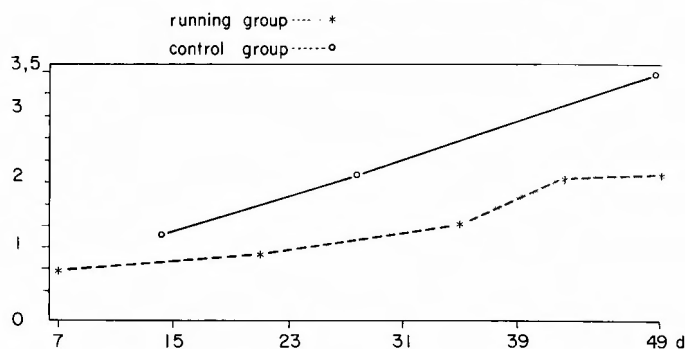


Fig. 5. ascent of tibial growth in loaded group in accordance to control group (1.2 mm x-ray-unit).

plate was after 11000 m decreased and the thickness of tibial epiphyseal plate is obvious below the control value (Fig. 3). Because of this we measured the tibial length once a week and the growth conduct of running animals covered a distance over 11000 m was compared with the same aged controls. The right tibial length of unloaded controls was 73% greater than in loaded animals after 11000 m and 55 days observation. The ascent of tibial growth in controls was steeper as in loaded animals (Figure 5). In loaded animals was no any abrupt loss of weight to recognize.

Electron Micrography:

The Golgi field was significant ($p < 0.05$) enlarged after 1000 m and between 3000 m and 8000 m almost normalized. After 11000 m it was significant less ($p < 0.05$) than in controls. The individual volumes of mitochondria were increased after 1000 m, but the number of them was deminishing between 1000 m and 3000 m. From 5400 m to 8000 m the number was increasing and the volume too. After 11000 m the number as well as the mitochondrial volume was decreased.

Fat vacuoles were significant deminished ($p < 0.05$) after 1000 m. After 11000 m the lipid content⁹ was double as in 1000 m.

Discussion

Dosed and chronic intensification of loading provokes rise of metabolic activity and production of proteoglycans and collagen, because of receptor capacity of chondrocytes without attended enlargement of collagenous network^{4, 6, 12}.

The secretory activity of the chondrocytes increases already after a short time of physical exercise, as we measured in enlargement of Golgi fields and increased number of Dictyosomes. The cartilage osmotic pressure increases because of hydrophilous swelling potential of glycosaminoglycans up to a limit after the collagenous structure is relax or begins to burst. Under continous increase of volumetric density of intercellular substance because of hypermetabolism the cartilage is getting elastic hardness respectively stiffness. Save that the collagenous fibres can burst because of overpressure, the fibrous network can break after stimulation of lysosomal

enzymes released from chondrocytes into the intercellular space²¹. Thereafter exists the possibility for enforced inhibition of water whereby the cartilage is losing stiffness, mechanical destruction will be possible and the cartilage internal system decompensates into a catabolic phase. According to this described conduct the volumetric density of intercellular substance is increasing significant in both evaluated zones of articular cartilage accompanied with relative increase of cartilage thickness and reduction of numeric density and mean cellvolume as features of adaptation under initial physical stress. In further loading the reduction of intercellular substance and cartilage thickness accompanied with relative increase of numeric density seems to be a compensative mechanism of cartilage internal system, what may prevent the burst of collagenous fibres and the pathologic hydration.

In radial zone the number and volume of mitochondria and volumetric density of fat vakuoles is decreased with the reduction of Golgi apparatus in this stable compensation- or plateau phase between 3000 m and 5400 m. Therefore we can find a decreased mean cellvolume of radial chondrocytes. In consequence it can be also a result of increased loading, cartilage thickness rise and deterioration of diffusional nourishment, therefore the chondrocytes are not able to work at an high level. The adaptation was treated fully. In this phase we can observe the consequent and absolute increase of cellvolume as cell hypertrophy in tangential zone. WEISS et al. 1972²⁶) described early osteoarthrotic changes in human cartilage also with notably enlargement of cells in superficial zone as feature of a metabolically active cell and evidence for increased Matrix density.

This rather abrupt increase in cell size may be firstly a compensation of the reduced metabolic activity in radial chondrocytes. But secondarily it would be rather due to cellular swelling, since the increase in number and volumes of cytoplasmic components alone could not account for this enlargement and it would be possible, that after hydration changed metabolites¹²) from radial zone effect a negative feed back on cells of superficial layers³¹). Cellular swelling is indicative for cellular degeneration. It seems that the osmotic pressure achieved the maximum so that the increased imbibition of water is apparently and fluid is drawn into cell like a hydrophic degeneration. After 5400 m up to 11000 m we can observe a phase of decompensation, because the cartilage thickness returns to the controlvalue and cellnumber and -volume are absolute decreased in tangential zone, what may be the consequence and an argument for preceded hydrophic degeneration. Therefore the intercellular substance is relative increased. The intracellular substance in radial zone is increased in resembling way, although the activity of Golgi apparatus is fallen below the controlvalue. It seems to be a result of enforced fluid of water into the intercellular space. At this point we suggest the first deterioration of the collagenous network. The absolute increase of numeric density in radial zone untill the controlvalue seemed to be a compensative mechanism for recreation of the constant cellnumber per area unit because of superficial cell reduction²⁰) and the attended further diminishing of cellvolume as feature of degeneration. This will be proved by measured reduction of number and volume of cytoplasmic components like Golgi apparatus and mitochondria. The absolute increase of fat vakuoles is seemed as a feature of regressive metabolic disturbances and lack of oxygen like in meniscus was

seen after overloading²⁷⁾ and in the other way the behaviour of intracellular lipid may underline the important role of stored fatty acids in changed metabolism of energy³¹⁾. In experience of the significant evaluated increase of cartilage thickness in central location of femoral condyle uncovered by meniscus we suggest this location as region of high stress gradient^{3, 11, 12, 18, 19)} with apparent adaptive reaction compared with the periphery. Therefore we can observe in this central location the significant small superficial cellular density with the cellular ratio equalizing significant high cellular density of radial zone. The mean cellvolume in tangential as well as radial zone were decreasing while the conduct of loading, but the volumina ratio between central and peripheric localized chondrocytes was turned to the central localized chondrocytes, what is seemed as functional feature of most apparent demands upon the metabolism in this region^{3, 12)} and let us conclude, that the meniscus confers a protective function on the underlaying articular cartilage.

Appearances like narrowing of tibial epiphyseal plate and reduction of numeric density in tibial as well as femoral proliferative zone with attended delayed skeletal growth of tibia seemed to be firstly due to undernourishment like other authors observed¹⁷⁾ especially in immature guinea pigs. But the nourishment both groups of animals received was sufficient and not vitamin C restricted¹⁶⁾, so that typical morphological findings of juvenile scurvey¹⁴⁾ could be excluded. We can suggest that the growth cartilage is exposed to the same loading conditions and reacts in resembling mode with degenerative changes as well as articular cartilage when the limit of tolerance is exceeded, what will have a clinical relevance in case of "overuse" of immature joints¹⁵⁾.

Conclusion

In summary, these results suggest that the form of mechanical loading used in these experiments caused a physiologic cycle of: adaptation—compensation—critic phase—decompensation, with overlaped transition into the pathologic range already in stage of decompensation.

The articular and growth cartilage of immature guinea pigs have the capacity to respond to mechanical stress within a range of tolerance. The tibial length difference suggest that physical forces may have a trophic effect upon proliferating cartilage as originally suggested by HUETER-VOLKMANN^{7, 9, 25)} and Arndt-Schulz-principle^{1, 7)}.

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References

- 1) Baum KV: Das biologische Grundgesetz (Arndt-Schulz'sches Gesetz). *Sportarzt* **9**: 209, 235, 257, 1963.
- 2) Dingle JT: The role of lysosomal enzymes in sceletal tissues. *J. Bone Joint Surg* **55B**: 87-95, 1973.
- 3) Hehne J, Ch Oelze, et al: Morphometrische Analyse des Tibiakopfes als Beitrag zum Arthroseproblem. *Z Orthop* **119**: 449-454, 1981.

- 4) Kampen GPJ, v JP Veldhuijzen, et al: Cartilage response to mechanic force in high-density chondrocyte culture *Arthritis Rheum* **28**: 419-424, 1985.
- 5) Kincaid SA and DC Van Sickle: Effects of exercise on the histochemical changes of articular chondrocytes in adult dogs. *J Am Vet Res* **43**: 1218-1226, 1982.
- 6) Mankin H and A Trasher: Water content and binding in normal and osteoarthritic human cartilage. *J Bone Joint Surg* **57**: 76-80, 1975.
- 7) Mau H: Spezifizierung der korrespondierenden Wachstums-Gesetze von Hueter-Volkman und Pauwels (Wachstumsdeformaten) und ihre Beziehungen zu den Belastungsdeformaten. *Z Orthop* **122**: 293-298, 1984.
- 8) McDevitt C and H Muir: Biochemical changes in the cartilage of the knee in experimental and natural osteoarthritis in the dog. *J Bone Joint Surg* **59B**: 94-100, 1976.
- 9) McMaster JH and CR Weinert: Effects of mechanical forces on growing cartilage. *Clin Orthop* **72**: 308-314, 1970.
- 10) Moskowitz R, D Howell, et al: Cartilage proteoglycan alterations in an experimentally induced model of rabbit osteoarthritis. *Arthritis Rheum* **22**: 155-163, 1979.
- 11) Oberlander W: Die Knorpeldickenverteilung in den Atlantooccipitalgelenken als Ausdruck ihrer Beanspruchung. 7. Europäischer Anatomen-Kongress, Innsbruck 5.9. 1984.
- 12) Otte P: Ätiologische und pathogenetische Vorstellungen bei der Arthrose. *Z Rheumatol* **42**: 311-321, 1983.
- 13) Pond MJ and G Nuki: Experimental induced osteoarthritis in the dog. *Ann. Rheumatic Dis* **32**: 387-388, 1973.
- 14) Resnick D and G Niwayama: Articular anatomy and histology In "Diagnosis of Bone and Joint Disorders", Vol. 2., W.B. Saunders—Philadelphia, London, Toronto: 2389-2391, 1983.
- 15) Roy S, R Steven, et al: Stress changes of the distal radial epiphyses in young gymnasts—A report of twenty one cases and a review of the literature, *Am J Sports Med* **13**: 301-308, 1985.
- 16) Schwartz ER, CR Leveille, et al: Proteoglycan structure and metabolism in normal and osteoarthrotic cartilage of guinea pig, *Arthritis Rheum* **24**: 1528-1539, 1981.
- 17) Silberberg M and R Silberberg: Changes in cartilage and bone of immature female guinea pigs due to undernourishment. *Arch Path* **30**: 675-688, 1940.
- 18) Silverstein E and L Sokoloff: Natural history of degenerative joint disease in small laboratory animals. 5. Osteoarthritis in guinea, *Arth Rheumatol* **1**: 82-86, 1958.
- 19) Slowman S and KD Brandt: Composition and glycosaminoglycan metabolism of articular cartilage from habitually loaded and habitually unloaded sites, *Arthritis Rheum* **29**: 88-94, 1986.
- 20) Stockwell RA: The interrelationship of cell density and cartilage thickness in mammalian articular cartilage. *J Anat* **109**: 411-421, 1971.
- 21) Stockwell RA, MEJ Billingham, et al: Ultrastructural changes in articular cartilage after experimental section of the anterior cruciate ligament in the dog, *J Anat* **136**: 425-439, 1983.
- 22) Stofft E and Ewen B: Hyaliner Gelenkknorpel im Tierexperiment, *Verh Anat Ges Jena* **74**: 317-325, 1980.
- 23) Stofft E and J Graf: Rasterelektronenmikroskopische Untersuchungen des hyalinen Gelenkknorpels, *Acta Anat* **116**, 114-125, 1983.
- 24) Underwood EE: A standardized system of notation stereologists., *Stereologia* **3**: 5-7, 1964.
- 25) Volkman Rv: Die Krankheiten der Bewegungsorgane. In "Pitha-Billroth" Handbuch der allgemeinen und speziellen Chirurgie, 2. Bd., 2. Abtlg., Enke, Stuttgart: 694., 1882.
- 26) Weiss Ch and S Mirow: An ultrastructural study of osteoarthritic changes in the articular cartilage of human knees, *J Bone Joint Surg* **54A**: 954-972, 1972.
- 27) Zollinger HU: Pathologische Anatomie, 1. Bd. Georg Thieme Verlag Stuttgart, 1982.

和文抄録

幼若モルモットにおける運動負荷が関節軟骨におよぼす効果
—組織学的計測と電顕的観察—

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V. Frankenberg, E., Engelmann, L., Zippel, H.

幼若モルモットの膝関節を用いて、運動負荷による関節軟骨および成長軟骨の早期変化を形態学的に研究した。

関節軟骨において細胞間物質と軟骨の厚さは、少量の運動負荷においては増加を示し、運動負荷の増加とともに減少した。11000 m 歩行の運動負荷においては、急に細胞間物質の増加と、軟骨の厚さの減少、関節軟

骨表層の細胞数、細胞の大きさの減少および深層における細胞の大きさの減少がみられた。

一方、成長軟骨においては、11000 m の歩行負荷において軟骨層の厚さの減少および脛骨長の減少が明らかであった。

以上の結果より、幼若軟骨は許容範囲内においては、運動負荷に対し形態学的に反応する能力を有している。